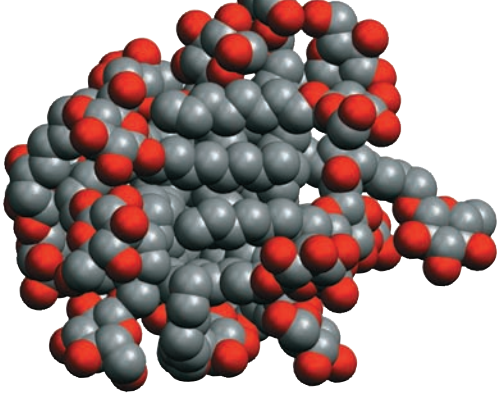


CHAPTER 2 Aqueous Solutions

1 Properties of Water
- A. Structure and Interactions
- B. Water as a Solvent
- C. Proton Mobility

2 Acids, Bases, and Buffers
- A. Acid–Base Reactions
- B. Buffers
- C. Polyprotic Acids

Life, as we know it, occurs in aqueous solution. Indeed, terrestrial life apparently arose in some primordial sea (Section 1-5B) and, as the fossil record indicates, did not venture onto dry land until comparatively recent times. Yet even those organisms that did develop the capacity to live out of water still carry the ocean with them: The compositions of their intracellular and extracellular fluids are remarkably similar to that of seawater. This is true even of organisms that live in such unusual environments as saturated brine, acidic hot sulfur springs, and petroleum.

Water is so familiar, we generally consider it to be a rather bland fluid of simple character. It is, however, a chemically reactive liquid with such extraordinary physical properties that, if chemists had discovered it in recent times, it would undoubtedly have been classified as an exotic substance.

The properties of water are of profound biological significance. *The structures of the molecules on which life is based—proteins, nucleic acids, lipids, and complex carbohydrates—result directly from their interactions with their aqueous environment. The combination of solvent properties responsible for the intramolecular and intermolecular associations of these substances is peculiar to water; no other solvent even resembles water in this respect.* Although the hypothesis that life could be based on organic polymers other than proteins and nucleic acids seems plausible, it is all but inconceivable that the complex structural organization and chemistry of living systems could exist in other than an aqueous medium. Indeed, direct observations on the surface of Mars, the only other planet in the solar system with temperatures compatible with life, indicate that it is presently devoid of both water and life.

Biological structures and processes can only be understood in terms of the physical and chemical properties of water. We therefore begin this chapter with a discussion of the molecular and solvent properties of water. In the following section we review its chemical behavior, that is, the nature of aqueous acids and bases.

1 PROPERTIES OF WATER

Water's peculiar physical and solvent properties stem largely from its extraordinary internal cohesiveness compared to that of almost any other liquid. In this section, we explore the physical basis of this phenomenon.

A. *Structure and Interactions*

The H_2O molecule has a bent geometry with an O—H bond distance of 0.958 Å and an H—O—H bond angle of 104.5° (Fig. 2-1). The large electronegativity differenc between H and O confers a 33% ionic character on the O—H bond as is indicated by water's dipole moment of 1.85 debye units. Water is clearly a highly polar molecule, a phenomenon with enormous implications for living systems.

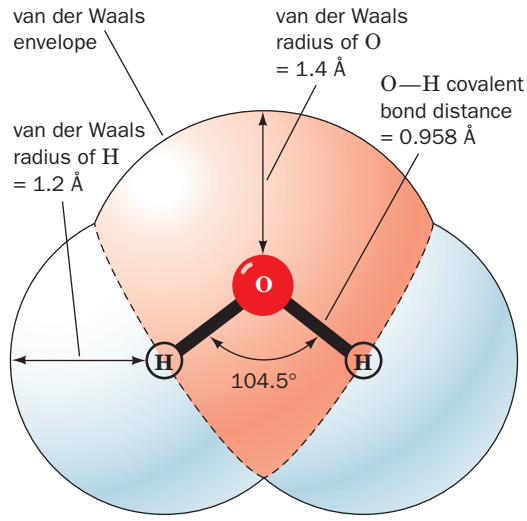


Figure 2-1 **Structure of the water molecule.** The outline represents the van der Waals envelope of the molecule (where the attractive components of the van der Waals interactions balance the repulsive components). The skeletal model of the molecule indicates its covalent bonds.

a. Water Molecules Associate Through Hydrogen Bonds

The electrostatic attractions between the dipoles of two water molecules tend to orient them such that the O—H bond on one water molecule points toward a lone-pair electron cloud on the oxygen atom of the other water molecule. This results in a directional intermolecular association known as a **hydrogen bond** (Fig. 2-2), an interaction that is crucial both to the properties of water itself and to its role as a biochemical solvent. In general, *a hydrogen bond may be represented as D—H···A , where D—H is a weakly acidic "donor group" such as N—H or O—H, and A is a lone-pair-bearing and thus weakly basic "acceptor atom" such as N or O*. Hence, a hydrogen bond is better represented as $^{\delta-}$D—H$^{\delta+}\cdots{}^{\delta-}$A, where the charge separation in the D—H bond arises from the greater electronegativity of D relative to H. The peculiar requirement of a central hydrogen atom rather than some other atom in a hydrogen bond stems from the hydrogen atom's small size: Only a hydrogen nucleus can approach the lone-pair electron cloud of an acceptor atom closely enough to permit an electrostatic association of significant magnitude. Moreover, as X-ray scattering measurements have revealed, hydrogen bonds are partially (~10%) covalent in character.

Hydrogen bonds are structurally characterized by an H···A distance that is at least 0.5 Å shorter than the calculated van der Waals distance (distance of closest approach between two nonbonded atoms) between the atoms. In water, for example, the O···H hydrogen bond distance is ~1.8 Å versus 2.6 Å for the corresponding van der Waals distance. The energy of a hydrogen bond (~20 kJ · mol^{-1} in H_2O) is small compared to covalent bond energies (for instance, 460 kJ · mol^{-1} for an O—H covalent bond). Nevertheless, most biological molecules have so many hydrogen bonding groups that hydrogen bonding is of paramount importance in determining their three-dimensional structures and their intermolecular associations. Hydrogen bonding is further discussed in Section 8-4B.

b. The Physical Properties of Ice and Liquid Water Largely Result from Intermolecular Hydrogen Bonding

The structure of ice provides a striking example of the cumulative strength of many hydrogen bonds. X-ray and neutron diffraction studies have established that water molecules in ice are arranged in an unusually open structure. Each water molecule is tetrahedrally surrounded by four nearest neighbors to which it is hydrogen bonded (Fig. 2-3). In two of these hydrogen bonds the central H_2O molecule is the "donor," and in the other two it is the "acceptor." As a consequence of its open structure, water is one of the very few substances that expands on freezing (at 0°C, liquid water has a density of 1.00 g · mL^{-1}, whereas ice has a density of 0.92 g · mL^{-1}).

The expansion of water on freezing has overwhelming consequences for life on Earth. Suppose that water contracted on freezing, that is, became more dense rather than less dense. Ice would then sink to the bottoms of lakes and oceans rather than float. This ice would be insulated from the sun so that oceans, with the exception of a thin surface layer of liquid in warm weather, would be permanently frozen solid (the water at great depths even in tropical oceans is close to 4°C, its temperature of maximum density). The reflection of sunlight by these frozen oceans and their cooling effect on the atmosphere would ensure that land temperatures would also be much colder than at present; that is, Earth would have a permanent ice age. Furthermore, since life apparently evolved in the ocean, it seems unlikely that life could have developed at all if ice contracted on freezing.

Although the melting of ice is indicative of the cooperative collapse of its hydrogen bonded structure, hydrogen bonds between water molecules persist in the liquid state. The heat of sublimation of ice at 0°C is 46.9 kJ · mol^{-1}. Yet

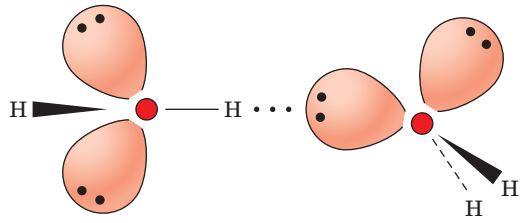


Figure 2-2 **Hydrogen bond between two water molecules.** The strength of this interaction is maximal when the O—H covalent bond points directly along a lone-pair electron cloud of the oxygen atom to which it is hydrogen bonded.

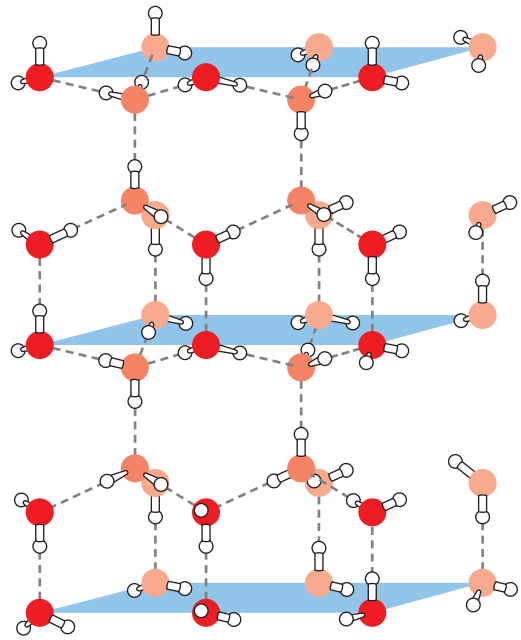


Figure 2-3 **Structure of ice.** The tetrahedral arrangement of the water molecules is a consequence of the roughly tetrahedral disposition of each oxygen atom's sp^3-hybridized bonding and lone-pair orbitals (Fig. 2-2). Oxygen and hydrogen atoms are represented, respectively, by red and white spheres, and hydrogen bonds are indicated by dashed lines. Note the open structure that gives ice its low density relative to liquid water. [After Pauling, L., *The Nature of the Chemical Bond* (3rd ed.), *p.* 465, Cornell University Press (1960).]

only ~6 kJ · mol^{-1} of this quantity can be attributed to the kinetic energy of gaseous water molecules. The remaining 41 kJ · mol^{-1} must therefore represent the energy required to disrupt the hydrogen bonding interactions holding an ice crystal together. The heat of fusion of ice (6.0 kJ · mol^{-1}) is ~15% of the energy required to disrupt the ice structure. *Liquid water is therefore only ~15% less hydrogen bonded than ice at 0°C.* Indeed, the boiling point of water is 264°C higher than that of methane (CH_4), a substance with nearly the same molecular mass as H_2O but which is incapable of hydrogen bonding (in the absence of intermolecular associations, substances with equal molecular masses should have similar boiling points). This reflects the extraordinary internal cohesiveness of liquid water resulting from its intermolecular hydrogen bonding.

c. Liquid Water Has a Rapidly Fluctuating Structure

X-ray and neutron scattering measurements of liquid water reveal a complex structure. Near 0°C, water exhibits an average nearest-neighbor O···O distance of 2.82 Å, which is slightly greater than the corresponding 2.76-Å distance in ice despite the greater density of the liquid. The X-ray data further indicate that each water molecule is surrounded by an average of about 4.4 nearest neighbors, which strongly suggests that the short-range structure of liquid water is predominantly tetrahedral in character. This picture is corroborated by the additional intermolecular distances in liquid water of around 4.5 and 7.0 Å, which are near the expected second and third nearest-neighbor distances in an icelike tetrahedral structure. Liquid water, however, also exhibits a 3.5-Å intermolecular distance, which cannot be rationalized in terms of an icelike structure. These average distances, moreover, become less sharply defined as the temperature increases into the physiologically significant range, thereby signaling the thermal breakdown of the short-range water structure.

The structure of liquid water is not simply described. This is because each water molecule reorients about once every 10^{-12} s, which makes the determination of water's instantaneous structure an experimentally and theoretically difficult problem (very few experimental techniques can make measurements over such short time spans). Indeed, only with the advent of modern computational methods have theoreticians felt that they are beginning to have a reasonable understanding of liquid water at the molecular level.

For the most part, molecules in liquid water are each hydrogen bonded to four nearest neighbors as they are in ice. These hydrogen bonds are distorted, however, so that the networks of linked molecules are irregular and varied, with the number of hydrogen bonds formed by each water molecule ranging from 3 to 6. Thus, for example, 3- to 7-membered rings of hydrogen bonded molecules commonly occur in liquid water (Fig. 2-4), in contrast to the cyclohexane-like 6-membered rings characteristic of ice (Fig. 2-3). Moreover, these networks are continually breaking up and re-forming over time periods on the order of 2×10^{-11} s. *Liquid water therefore consists of a rapidly fluctuating, space-filling network of hydrogen bonded H_2O molecules that, over short distances, resembles that of ice.*

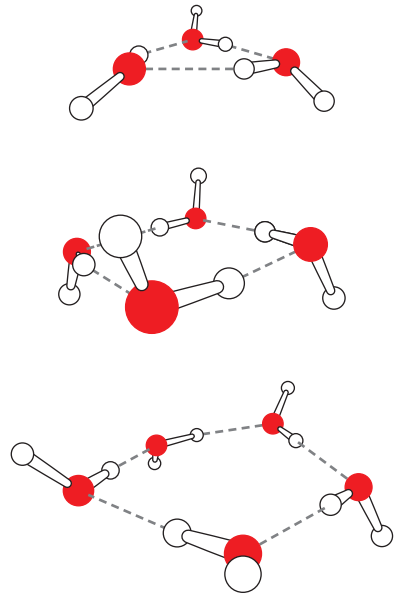


Figure 2-4 **Theoretically predicted and spectroscopically confirmed structures of the water trimer, tetramer, and pentamer.** Note that these rings are all essentially planar, with each water molecule acting as both a hydrogen bonding donor and acceptor and with the free hydrogens located above and below the planes of the rings. [After Liu, K., Cruzan, J.D., and Saykelly, R.J., *Science* **271,** 930 (1996).]

B. *Water as a Solvent*

Solubility depends on the ability of a solvent to interact with a solute more strongly than solute particles interact with each other. Water is said to be the "universal solvent." Although this statement cannot literally be true, water certainly dissolves more types of substances and in greater amounts than any other solvent. In particular, the polar character of water makes it an excellent solvent for polar and ionic materials, which are therefore said to be **hydrophilic** (Greek: *hydor,* water + *philos,* loving). On the other hand, nonpolar substances are virtually insoluble in water ("oil and water don't mix") and are consequently described as being **hydrophobic** (Greek: *phobos,* fear). Nonpolar substances, however, are soluble in nonpolar solvents such as CCl_4 or hexane. This information is summarized by another maxim, "like dissolves like."

Why do salts dissolve in water? Salts, such as NaCl or K_2HPO_4, are held together by ionic forces. The ions of a salt, as do any electrical charges, interact according to **Coulomb's law:**

$$F = \frac{kq_1q_2}{Dr^2} \qquad [2.1]$$

where F is the force between two electrical charges, q_1 and q_2, that are separated by the distance r, D is the **dielectric constant** of the medium between them, and k is a proportionality constant (8.99×10^9 J · m · C^{-2}). Thus, as the dielectric constant of a medium increases, the force between its embedded charges decreases; that is, the dielectric constant

Table 2-1 **Dielectric Constants and Permanent Molecular Dipole Moments of Some Common Solvents**

Substance	Dielectric Constant	Dipole Moment (debye)
Formamide	110.0	3.37
Water	78.5	1.85
Dimethyl sulfoxide	48.9	3.96
Methanol	32.6	1.66
Ethanol	24.3	1.68
Acetone	20.7	2.72
Ammonia	16.9	1.47
Chloroform	4.8	1.15
Diethyl ether	4.3	1.15
Benzene	2.3	0.00
Carbon tetrachloride	2.2	0.00
Hexane	1.9	0.00

Source: Brey, W.S., *Physical Chemistry and Its Biological Applications, p.* 26, Academic Press (1978).

of a solvent is a measure of its ability to keep opposite charges apart. In a vacuum, D is unity and in air, it is only negligibly larger. The dielectric constants of several common solvents, together with their permanent molecular dipole moments, are listed in Table 2-1. Note that these quantities tend to increase together, although not in a regular way.

The dielectric constant of water is among the highest of any pure liquid, whereas those of nonpolar substances, such as hydrocarbons, are relatively small. The force between two ions separated by a given distance in nonpolar liquids such as hexane or benzene is therefore 30 to 40 times greater than that in water. Consequently, in nonpolar solvents (low D), ions of opposite charge attract each other so strongly that they coalesce to form a salt, whereas the much weaker forces between ions in water solution (high D) permit significant quantities of the ions to remain separated.

An ion immersed in a polar solvent attracts the oppositely charged ends of the solvent dipoles, as is diagrammed in Fig. 2-5 for water. The ion is thereby surrounded by several concentric shells of oriented solvent molecules. Such ions are said to be **solvated** or, if water is the solvent, to be **hydrated.** The electric field produced by the solvent dipoles opposes that of the ion so that, in effect, the ionic charge is spread over the volume of the solvated complex. This arrangement greatly attenuates the coulombic forces between ions, which is why polar solvents have such high dielectric constants.

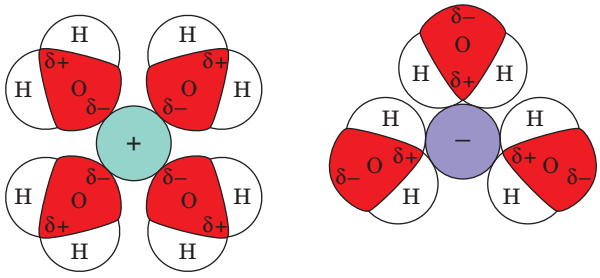


Figure 2-5 **Solvation of ions by oriented water molecules.**

The orienting effect of ionic charges on dipolar molecules is opposed by thermal motions, which continually tend to randomly reorient all molecules. The dipoles in a solvated complex are therefore only partially oriented. The reason why the dielectric constant of water is so much greater than that of other liquids with comparable dipole moments is that liquid water's hydrogen bonded structure permits it to form oriented structures that resist thermal randomization, thereby more effectively distributing ionic charges. Indeed, ice under high pressure has a measured dielectric constant of 3 because its water molecules cannot reorient in response to an external electric field.

The bond dipoles of uncharged polar molecules make them soluble in aqueous solutions for the same reasons that ionic substances are water soluble. The solubilities of polar and ionic substances are enhanced if they carry functional groups, such as hydroxyl (—OH), keto (—C═O), carboxyl (—CO_2H or —COOH), or amino (—NH_2) groups, that can form hydrogen bonds with water, as is illustrated in Fig. 2-6. Indeed, water-soluble biomolecules such as proteins, nucleic acids, and carbohydrates bristle with just such groups. Nonpolar substances, in contrast, lack both hydrogen bonding donor and acceptor groups.

a. Amphiphiles Form Micelles and Bilayers

Most biological molecules have both polar (or ionically charged) and nonpolar segments and are therefore simultaneously hydrophilic and hydrophobic. Such molecules, for

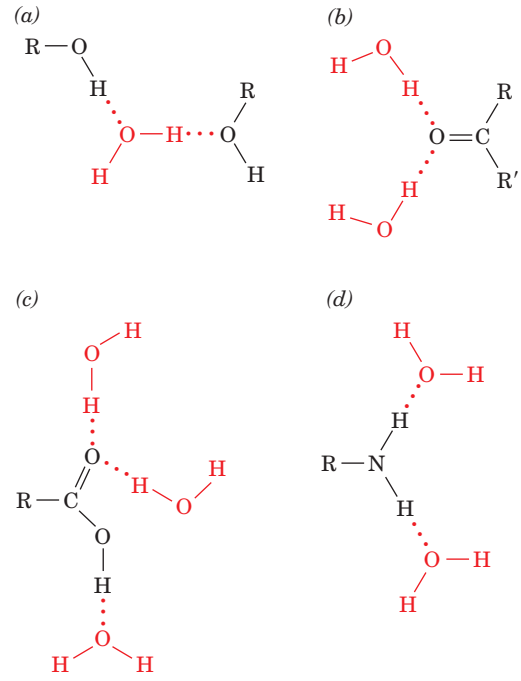


Figure 2-6 **Hydrogen bonding by functional groups.** Hydrogen bonds form between water and (*a*) hydroxyl groups, (*b*) keto groups, (*c*) carboxyl groups, and (*d*) amino groups.

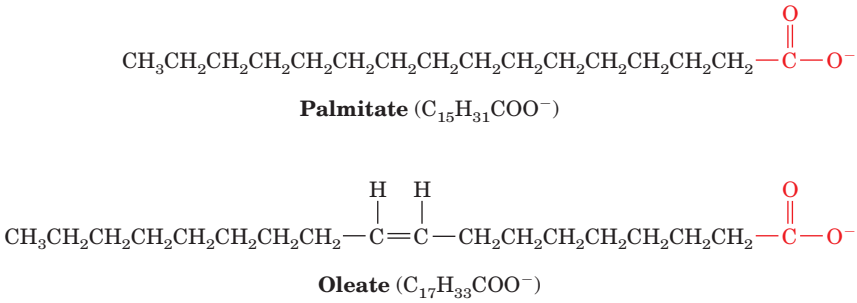


Figure 2-7 **Examples of fatty acid anions.** They consist of a polar carboxylate group coupled to a long nonpolar hydrocarbon chain.

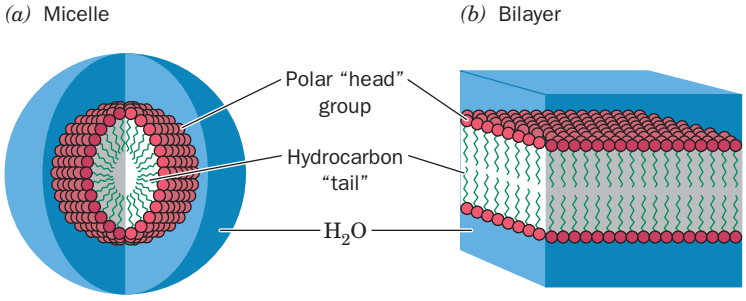


Figure 2-8 **Associations of amphipathic molecules in aqueous solutions.** The polar "head" groups are hydrated, whereas the nonpolar "tails" aggregate so as to exclude the aqueous solution. (*a*) A spheroidal aggregate of amphipathic molecules known as a micelle. (*b*) An extended planar aggregate of amphipathic molecules called a **bilayer.** The bilayer may form a closed spheroidal shell, known as a vesicle, that encloses a small amount of aqueous solution.

example, **fatty acid** ions (soap ions; Fig. 2-7), are said to be **amphiphilic** or, synonymously, **amphipathic** (Greek: *amphi,* both + *pathos,* passion). How do amphiphiles interact with an aqueous solvent? Water, of course, tends to hydrate the hydrophilic portion of an amphiphile, but it also tends to exclude its hydrophobic portion. Amphiphiles consequently tend to form water-dispersed structurally ordered aggregates. Such aggregates may take the form of **micelles,** which are globules of up to several thousand amphiphiles arranged with their hydrophilic groups at the globule surface so that they can interact with the aqueous solvent while the hydrophobic groups associate at the center so as to exclude solvent (Fig. 2-8*a*). However, the model drawn in Fig. 2-8*a* is an oversimplification because it is geometrically impossible for all the hydrophobic groups to occupy the center of the micelle. Instead, the amphipilic molecules pack in a more disorganized and rapidly fluctuating fashion that largely buries their hydrophobic groups and exposes their polar groups (Fig. 2-9). Alternatively, amphiphiles may arrange themselves to form bilayered sheets or vesicles (Fig. 2-8*b*) in which the polar groups face the aqueous phase.

The interactions stabilizing a micelle or bilayer are collectively described as **hydrophobic forces** or **hydrophobic interactions** to indicate that they result from the tendency of water to exclude hydrophobic groups. Hydrophobic interactions are relatively weak compared to hydrogen bonds and lack directionality. Nevertheless, hydrophobic interactions are of pivotal biological importance because, as we shall see in later chapters, they are largely responsible for the structural integrity of biological macromolecules (Sections 8-4C and 29-2C), as well as that of supramolecular aggregates such as membranes. Note that hydrophobic interactions are peculiar to an aqueous environment. Other polar solvents do not promote such associations.

C. *Proton Mobility*

When an electrical current is passed through an ionic solution, the ions migrate toward the electrode of opposite polarity at a rate proportional to the electrical field and inversely proportional to the frictional drag experienced by the ion as it moves through the solution. This latter quantity, as Table 2-2 indicates, varies with the size of the ion. Note, however, that the ionic mobilities of both H_3O^+ and

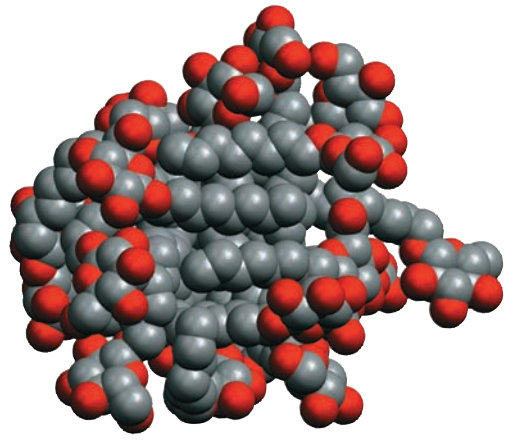


Figure 2-9 **Model of a micelle.** Twenty molecules of octyl glucoside (an eight-carbon chain with a sugar head group) are shown in space-filling form in this computer-generated model. The molecules' polar O atoms are red and the C atoms are gray. H atoms have been omitted for clarity. Computer simulations indicate that such micelles have an irregular, rapidly fluctuating structure (unlike the symmetric aggregate pictured in Fig. 2-8*a*) such that portions of the hydrophobic tails are exposed on the micelle surface at any given instant. [Courtesy of Michael Garavito and Shelagh Ferguson-Miller, Michigan State University.]

Table 2.2 **Ionic Mobilities[a] in H_2O at 25°C**

Ion	Mobility $\times 10^{-5}$ ($cm^2 \cdot V^{-1} \cdot s^{-1}$)
H_3O^+	362.4
Li^+	40.1
Na^+	51.9
K^+	76.1
NH_4^+	76.0
Mg^{2+}	55.0
Ca^{2+}	61.6
OH^-	197.6
Cl^-	76.3
Br^-	78.3
CH_3COO^-	40.9
SO_4^{2-}	79.8

[a] Ionic mobility is the distance an ion moves in 1 s under the influence of an electric field of 1 $V \cdot cm^{-1}$.

Source: Brey, W.S., *Physical Chemistry and Its Biological Applications, p.* 172, Academic Press (1978).

OH^- are anomalously large compared to those of other ions. For H_3O^+ (the **hydronium ion,** which is abbreviated H^+; a bare proton has no stable existence in aqueous solution), this high migration rate results from the ability of protons to jump rapidly from one water molecule to another, as is diagrammed in Fig. 2-10. Although a given hydronium ion can physically migrate through solution in the manner of, say, an Na^+ ion, the rapidity of the proton-jump mechanism makes the H_3O^+ ion's effective ionic mobility much greater than it otherwise would be (the mean lifetime of a given H_3O^+ ion is 10^{-12} s at 25°C). The anomalously high ionic mobility of the OH^- ion is likewise accounted for by the proton-jump mechanism but, in this case, the apparent direction of ionic migration is opposite

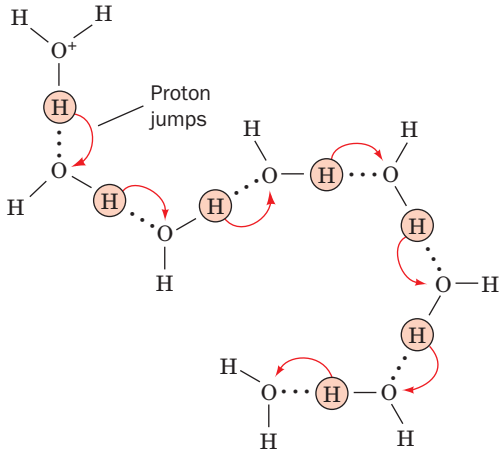


Figure 2-10 **Mechanism of hydronium ion migration in aqueous solution via proton jumps.** Proton jumps, which mostly occur at random, take place rapidly compared with direct molecular migration, thereby accounting for the observed high ionic mobilities of hydronium and hydroxyl ions in aqueous solutions.

to the direction of proton jumping. Proton jumping is also responsible for the observation that *acid–base reactions are among the fastest reactions that take place in aqueous solutions,* and as we shall see (Section 23-3B), is of importance in biological proton-transfer reactions.

2 ACIDS, BASES, AND BUFFERS

Biological molecules, such as proteins and nucleic acids, bear numerous functional groups, such as carboxyl and amino groups, that can undergo acid–base reactions. Many properties of these molecules therefore vary with the acidities of the solutions in which they are immersed. In this section we discuss the nature of acid–base reactions and how acidities are controlled, both physiologically and in the laboratory.

A. *Acid–Base Reactions*

Acids and **bases,** in a definition coined in the 1880s by Svante Arrhenius, are, respectively, substances capable of donating protons and hydroxide ions. This definition is rather limited, because, for example, it does not account for the observation that NH_3, which lacks an OH^- group, exhibits basic properties. In a more general definition, which was formulated in 1923 by Johannes Brønsted and Thomas Lowry, *an acid is a substance that can donate protons (as in the Arrhenius definition) and a base is a substance that can accept protons.* Under this definition, in every acid–base reaction,

$$HA + H_2O \rightleftharpoons H_3O^+ + A^-$$

a **Brønsted acid** (here HA) reacts with a **Brønsted base** (here H_2O) to form the **conjugate base** of the acid (A^-) and the **conjugate acid** of the base (H_3O^+) (this reaction is usually abbreviated $HA \rightleftharpoons H^+ + A^-$ with the participation of H_2O implied). Accordingly, the acetate ion (CH_3COO^-) is the conjugate base of acetic acid (CH_3COOH) and the ammonium ion (NH_4^+) is the conjugate acid of ammonia (NH_3). (In a yet more general definition of acids and bases, Gilbert Lewis described a **Lewis acid** as a substance that can accept an electron pair and a **Lewis base** as a substance that can donate an electron pair. This definition, which is applicable to both aqueous and nonaqueous systems, is unnecessarily broad for describing most biochemical phenomena.)

a. The Strength of an Acid Is Specified by Its Dissociation Constant

The above acid dissociation reaction is characterized by its **equilibrium constant,** which, for acid–base reactions, is known as a **dissociation constant,**

$$K = \frac{[H_3O^+][A^-]}{[HA][H_2O]} \qquad [2.2]$$

a quantity that is a measure of the relative proton affinities of the HA/A^- and H_3O^+/H_2O conjugate acid–base pairs. Here, as throughout the text, quantities in square brackets symbolize the molar concentrations of the

enclosed substances. Since in dilute aqueous solutions the water concentration is essentially constant with $[H_2O] = 1000\ g \cdot L^{-1}/18.015\ g \cdot mol^{-1} = 55.5\ M$, this term is customarily combined with the dissociation constant, which then takes the form

$$K_a = K[H_2O] = \frac{[H^+][A^-]}{[HA]} \qquad [2.3]$$

For brevity, however, we shall henceforth omit the subscript "*a*." The dissociation constants for acids useful in preparing biochemical solutions are listed in Table 2-3.

Acids may be classified according to their relative strengths, that is, according to their abilities to transfer a proton to water. Acids with dissociation constants smaller than that of H_3O^+ (which, by definition, is unity in aqueous solutions) are only partially ionized in aqueous solutions and are known are **weak acids** ($K < 1$). Conversely, **strong acids** have dissociation constants larger than that of H_3O^+ so that they are almost completely ionized in aqueous solutions ($K > 1$). The acids listed in Table 2-3 are all weak acids. However, many of the so-called mineral acids, such as $HClO_4$, HNO_3, HCl, and H_2SO_4 (for its first ionization), are strong acids. Since strong acids rapidly transfer all their protons to H_2O, the strongest acid that can stably exist in aqueous solutions is H_3O^+. Likewise, there can be no stronger base in aqueous solutions than OH^-.

Table 2-3 **Dissociation Constants and p*K*'s at 25°C of Some Acids in Common Laboratory Use as Biochemical Buffers**

Acid	*K* (*M*)	p*K*
Oxalic acid	5.37×10^{-2}	1.27 (pK_1)
H_3PO_4	7.08×10^{-3}	2.15 (pK_1)
Citric acid	7.41×10^{-4}	3.13 (pK_1)
Formic acid	1.78×10^{-4}	3.75
Succinic acid	6.17×10^{-5}	4.21 (pK_1)
$Oxalate^-$	5.37×10^{-5}	4.27 (pK_2)
Acetic acid	1.74×10^{-5}	4.76
$Citrate^-$	1.74×10^{-5}	4.76 (pK_2)
$Citrate^{2-}$	3.98×10^{-6}	5.40 (pK_3)
$Succinate^-$	2.29×10^{-6}	5.64 (pK_2)
2-(*N*-Morpholino)ethanesulfonic acid (MES)	8.13×10^{-7}	6.09
Cacodylic acid	5.37×10^{-7}	6.27
H_2CO_3	4.47×10^{-7}	6.35 (pK_1)
N-(2-Acetamido)iminodiacetic acid (ADA)	2.69×10^{-7}	6.57
Piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES)	1.74×10^{-7}	6.76
N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES)	1.58×10^{-7}	6.80
$H_2PO_4^-$	1.51×10^{-7}	6.82 (pK_2)
3-(*N*-Morpholino)propanesulfonic acid (MOPS)	7.08×10^{-8}	7.15
N-2-Hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES)	3.39×10^{-8}	7.47
N-2-Hydroxyethylpiperazine-*N*′-3-propanesulfonic acid (HEPPS)	1.10×10^{-8}	7.96
N-[Tris(hydroxymethyl)methyl]glycine (Tricine)	8.91×10^{-9}	8.05
Tris(hydroxymethyl)aminomethane (Tris)	8.32×10^{-9}	8.08
Glycylglycine	5.62×10^{-9}	8.25
N,*N*-Bis(2-hydroxyethyl)glycine (Bicine)	5.50×10^{-9}	8.26
Boric acid	5.75×10^{-10}	9.24
NH_4^+	5.62×10^{-10}	9.25
Glycine	1.66×10^{-10}	9.78
HCO_3	4.68×10^{-11}	10.33 (pK_2)
Piperidine	7.58×10^{-12}	11.12
HPO_4^{2-}	4.17×10^{-13}	12.38 (pK_3)

Source: Mostly Dawson, R.M.C., Elliott, D.C., Elliott, W.H., and Jones, K.M., *Data for Biochemical Research* (3rd ed.), *pp.* 424–425, Oxford Science Publications (1986); *and* Good, N.E., Winget, G.D., Winter, W., Connolly, T.N., Izawa, S., and Singh, R.M.M., *Biochemistry* **5,** 467 (1966).

Water, being an acid, has a dissociation constant:

$$K = \frac{[H^+][OH^-]}{[H_2O]}$$

As above, the constant $[H_2O] = 55.5M$ can be incorporated into the dissociation constant to yield the expression for the ionization constant of water,

$$K_w = [H^+][OH^-] \qquad [2.4]$$

The value of K_w at 25°C is $10^{-14}\ M^2$. Pure water must contain equimolar amounts of H^+ and OH^- so that $[H^+] = [OH^-] = (K_w)^{1/2} = 10^{-7}\ M$. Since $[H^+]$ and $[OH^-]$ are reciprocally related by Eq. [2.4], if $[H^+]$ is greater than this value, $[OH^-]$ must be correspondingly less and vice versa. Solutions with $[H^+] = 10^{-7}\ M$ are said to be **neutral,** those with $[H^+] > 10^{-7}\ M$ are said to be **acidic,** and those with $[H^+] < 10^{-7}\ M$ are said to be **basic.** Most physiological solutions have hydrogen ion concentrations near neutrality. For example, human blood is normally slightly basic, with $[H^+] = 4.0 \times 10^{-8}\ M$.

The values of $[H^+]$ for most solutions are inconveniently small and difficult to compare. A more practical quantity, which was devised in 1909 by Søren Sørensen, is known as the **pH:**

$$pH = -\log[H^+] \qquad [2.5]$$

The pH of pure water is 7.0, whereas acidic solutions have pH < 7.0 and basic solutions have pH > 7.0. For a 1*M* solution of a strong acid, pH = 0 and for a 1*M* solution of a strong base, pH = 14. Note that if two solutions differ in pH by one unit, they differ in $[H^+]$ by a factor of 10. The pH of a solution may be accurately and easily determined through electrochemical measurements with a device known as a **pH meter.**

b. The pH of a Solution Is Determined by the Relative Concentrations of Acids and Bases

The relationship between the pH of a solution and the concentrations of an acid and its conjugate base can be easily derived by rearranging Eq. [2.3]

$$[H^+] = K\left(\frac{[HA]}{[A^-]}\right)$$

and substituting it into Eq. [2.5]

$$pH = -\log K + \log\left(\frac{[A^-]}{[HA]}\right)$$

Defining $pK = -\log K$ in analogy with Eq. [2.5], we obtain the **Henderson–Hasselbalch equation:**

$$pH = pK + \log\left(\frac{[A^-]}{[HA]}\right) \qquad [2.6]$$

This equation indicates that *the pK of an acid is numerically equal to the pH of the solution when the molar concentrations of the acid and its conjugate base are equal.* Table 2-3 lists the p*K* values of several acids.

B. *Buffers*

A 0.01-mL droplet of 1*M* HCl added to 1 L of pure water changes the water's pH from 7 to 5, which represents a 100-fold increase in $[H^+]$. Yet, since the properties of biological substances vary significantly with small changes in pH, they require environments in which the pH is insensitive to additions of acids or bases. To understand how this is possible, let us consider the titration of a weak acid with a strong base.

Figure 2-11 shows how the pH values of 1-L solutions of 1*M* acetic acid, $H_2PO_4^-$, and ammonium ion (NH_4^+), vary with the quantity of OH^- added. Titration curves such as those in Fig. 2-11, as well as distribution curves such as those

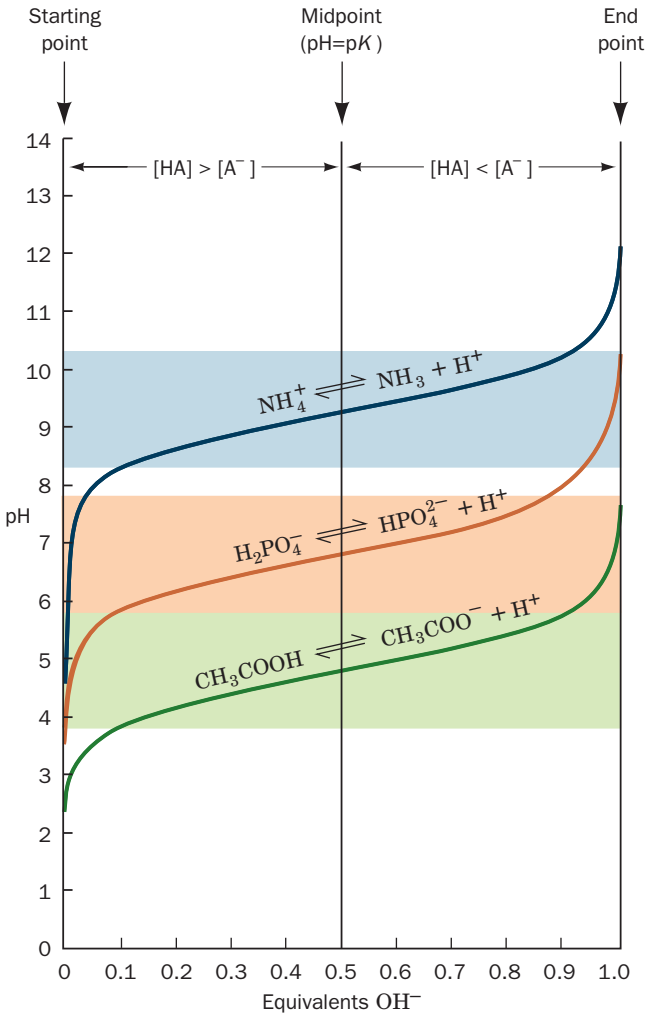


Figure 2-11 **Acid–base titration curves of 1-L solutions of 1*M* acetic acid, $H_2PO_4^-$, and NH_4^+ by a strong base.** At the starting point of each titration, the acid form of the conjugate acid–base pair overwhelmingly predominates. At the midpoint of the titration, where pH = p*K*, the concentration of the acid is equal to that of its conjugate base. Finally, at the end point of the titration, where the equivalents of strong base added equal the equivalents of acid at the starting point, the conjugate base is in great excess over acid. The shaded bands indicate the pH ranges over which the corresponding solution can function effectively as a buffer. **See the Animated Figures.**

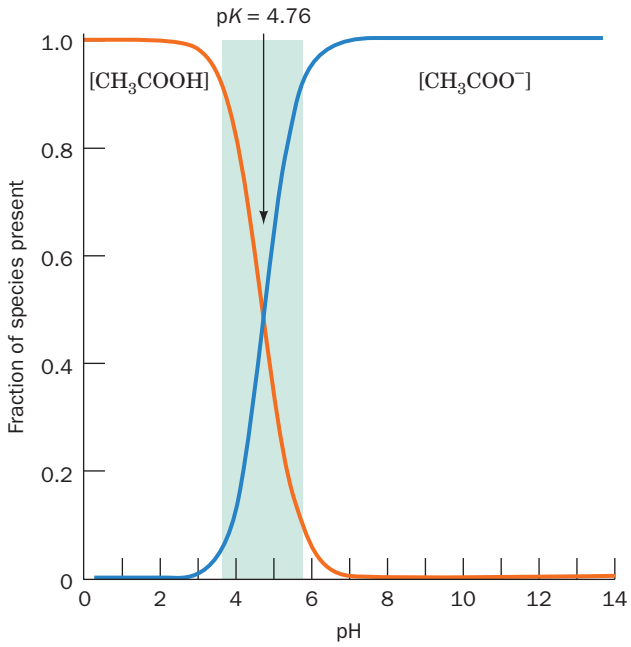


Figure 2-12 **Distribution curves for acetic acid and acetate ion.** The fraction of species present is given as the ratio of the concentration of CH_3COOH or CH_3COO^- to the total concentrations of these two species. The customarily accepted useful buffer range of p$K \pm 1$ is indicated by the shaded region.

in Fig. 2-12, may be calculated using the Henderson–Hasselbalch equation. Near the beginning of the titration, a significant fraction of the A^- present arises from the dissociation of HA. Similarly, near the end point, much of the HA derives from the reaction of A^- with H_2O. Throughout most of the titration, however, the OH^- added reacts essentially completely with the HA to form A^- so that

$$[A^-] = \frac{x}{V} \qquad [2.7]$$

where x represents the equivalents of OH^- added and V is the volume of the solution. Then, letting c_0 represent the equivalents of HA initially present,

$$[HA] = \frac{c_0 - x}{V} \qquad [2.8]$$

Incorporating these relationships into Eq. [2.6] yields

$$pH = pK + \log\left(\frac{x}{c_0 - x}\right) \qquad [2.9]$$

which accurately describes a titration curve except near its wings (these regions require more exact treatments that take into account the ionizations of water).

Several details about the titration curves in Fig. 2-11 should be noted:

1. The curves have similar shapes but are shifted vertically along the pH axis.

2. The pH at the **equivalence point** of each titration (where the equivalents of OH^- added equal the equivalents of HA initially present) is >7 because of the reaction of A^- with H_2O to form HA + OH^-; similarly, each initial pH is <7.

3. The pH at the midpoint of each titration is numerically equal to the pK of its corresponding acid; here, according to the Henderson–Hasselbalch equation, $[HA] = [A^-]$.

4. The slope of each titration curve is much less near its midpoint than it is near its wings. This indicates that *when $[HA] \approx [A^-]$, the pH of the solution is relatively insensitive to the addition of strong base or strong acid. Such a solution, which is known as an* ***acid–base buffer,*** *is resistant to pH changes because small amounts of added H^+ or OH^-, respectively, react with the A^- or HA present without greatly changing the value of $\log([A^-]/[HA])$.*

a. Buffers Stabilize a Solution's pH

The ability of a buffer to resist pH changes with added acid or base is directly proportional to the total concentration of the conjugate acid–base pair, $[HA] + [A^-]$. It is maximal when pH = pK and decreases rapidly with a change in pH from that point. A good rule of thumb is that *a weak acid is in its useful buffer range within 1 pH unit of its pK* (the shaded regions of Figs. 2-11 and 2-12). Above this range, where the ratio $[A^-]/[HA] > 10$, the pH of the solution changes rapidly with added strong base. A buffer is similarly impotent with addition of strong acid when its pK exceeds the pH by more than a unit.

Biological fluids, both those found intracellularly and extracellularly, are heavily buffered. For example, the pH of the blood in healthy individuals is closely controlled at pH 7.4. The phosphate and carbonate ions that are components of most biological fluids are important in this respect because they have pK's in this range (Table 2-3). Moreover, many biological molecules, such as proteins, nucleic acids, and lipids, as well as numerous small organic molecules, bear multiple acid–base groups that are effective as buffer components in the physiological pH range.

The concept that the properties of biological molecules vary with the acidity of the solution in which they are dissolved was not fully appreciated before the beginning of the twentieth century so that the acidities of biochemical preparations made before that time were rarely controlled. Consequently these early biochemical experiments yielded poorly reproducible results. More recently, biochemical preparations have been routinely buffered to simulate the properties of naturally occurring biological fluids. Many of the weak acids listed in Table 2-3 are commonly used as buffers in biochemical preparations. In practice, the chosen weak acid and one of its soluble salts are dissolved in the (nearly equal) mole ratio necessary to provide the desired pH and, with the aid of a pH meter, the resulting solution is fine-tuned by titration with strong acid or base.

C. *Polyprotic Acids*

Substances that bear more than one acid–base group, such as H_3PO_4 or H_2CO_3, as well as most biomolecules, are known as **polyprotic acids.** The titration curves of such sub-

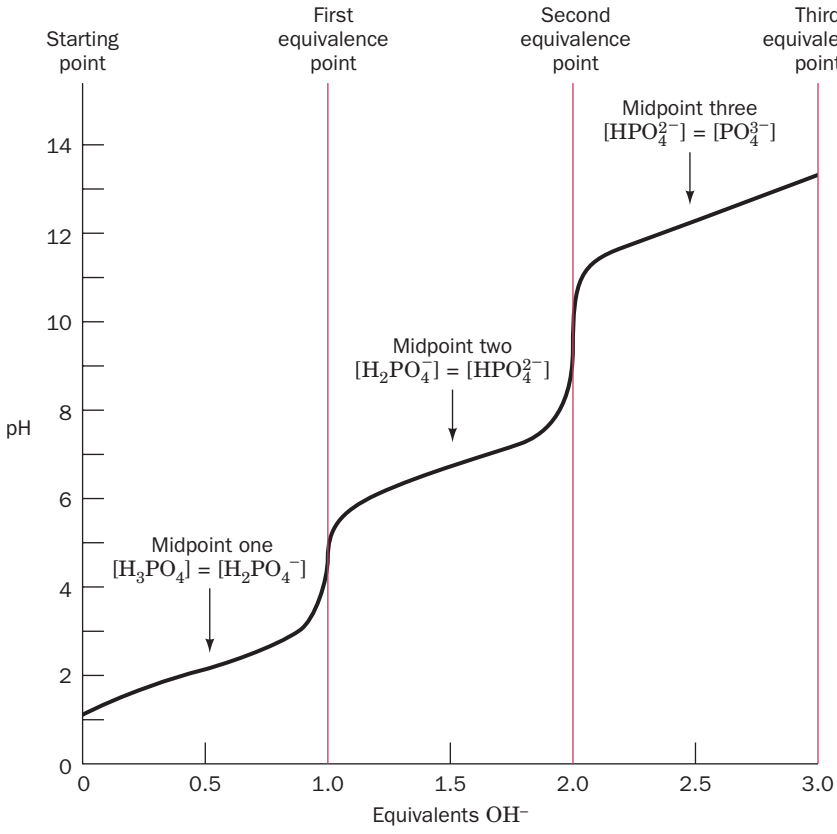
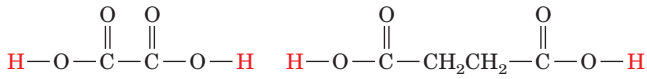


Figure 2-13 **Titration curve of a 1-L solution of 1*M* H_3PO_4.** The two intermediate equivalence points occur at the steepest parts of the curve. Note the flatness of the curve near its starting points and end points in comparison with the curved ends of the titration curves in Fig. 2-11. This indicates that H_3PO_4 (pK_1 = 2.15) is verging on being a strong acid and PO_4^{3-} (pK_3 = 12.38) is verging on being a strong base. **See the Animated Figures.**

stances, as is illustrated in Fig. 2-13 for H_3PO_4, are characterized by multiple p*K*'s, one for each ionization step. Exact calculations of the concentrations of the various ionic species present at a given pH is clearly a more complex task than for a **monoprotic acid.**

*The p*K*'s of two closely associated acid–base groups are not independent.* The ionic charge resulting from a proton dissociation electrostatically inhibits further proton dissociation from the same molecule, thereby increasing the values of the corresponding p*K*'s. This effect, according to Coulomb's law, decreases as the distance between the ionizing groups increases. For example, the p*K*'s of **oxalic acid's** two adjacent carboxyl groups differ by 3 pH units (Table 2-3), whereas those of **succinic acid,** in which the carboxyl groups are separated by two methylene groups, differ by 1.4 units.



Oxalic acid **Succinic acid**

Likewise, successive ionizations from the same center, such as in H_3PO_4 or H_2CO_3, have p*K*'s that differ by 4 to 5 pH units. If the p*K*'s for successive ionizations of a polyprotic acid differ by at least 3 pH units, it can be accurately assumed that, at a given pH, only the members of the conjugate acid–base pair characterized by the nearest p*K* are present in significant concentrations. This, of course, greatly simplifies the calculations for determining the concentrations of the various ionic species present.

a. Polyprotic Acids with Closely Spaced p*K*'s Have Molecular Ionization Constants

If the p*K*'s of a polyprotic acid differ by less than ~2 pH units, as is true in perhaps the majority of biomolecules, the ionization constants measured by titration are not true group ionization constants but, rather, reflect the average ionization of the groups involved. The resulting ionization constants are therefore known as **molecular ionization constants.**

Consider the acid–base equilibria shown in Fig. 2-14 in which there are two nonequivalent protonation sites. Here, the quantities K_A, K_B, K_C, and K_D, the ionization constants for each group, are alternatively called **microscopic ionization constants.** The molecular ionization constant for the removal of the first proton from HAH is

$$K_1 = \frac{[H^+]([AH^-] + [HA^-])}{[HAH]} = K_A + K_B \quad [2.10]$$

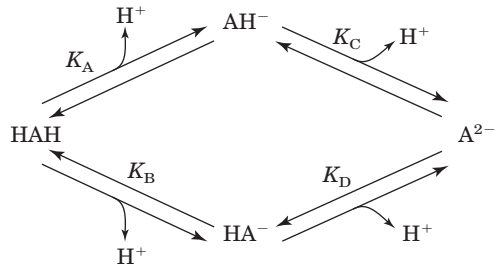


Figure 2-14 **Ionization of an acid that has two nonequivalent protonation sites.**

Similarly, the molecular ionization constant K_2 for the removal of the second proton is

$$K_2 = \frac{[H^+][A^{2-}]}{[AH^-] + [HA^-]} = \frac{1}{(1/K_C) + (1/K_D)}$$

$$= \frac{K_C K_D}{K_C + K_D} \qquad [2.11]$$

If $K_A \gg K_B$, then $K_1 \approx K_A$; that is, the first molecular ionization constant is equal to the microscopic ionization constant of the more acidic group. Likewise, if $K_D \gg K_C$, then $K_2 \approx K_C$, so that the second molecular ionization constant is the microscopic ionization constant of the less acidic group. If the ionization steps differ sufficiently in their pK's, the molecular ionization constants, as expected, become identical to the microscopic ionization constants.

CHAPTER SUMMARY

1 Properties of Water Water is an extraordinary substance, the properties of which are of great biological importance. A water molecule can simultaneously participate in as many as four hydrogen bonds: two as a donor and two as an acceptor. These hydrogen bonds are responsible for the open, low-density structure of ice. Much of this hydrogen bonded structure exists in the liquid phase, as is evidenced by the high boiling point of water compared to those of other substances of similar molecular masses. Physical and theoretical evidence indicates that liquid water maintains a rapidly fluctuating, hydrogen bonded molecular structure that, over short ranges, resembles that of ice. The unique solvent properties of water derive from its polarity as well as its hydrogen bonding properties. In aqueous solutions, ionic and polar substances are surrounded by multiple concentric hydration shells of oriented water dipoles that act to attenuate the electrostatic interactions between the charges in the solution. The thermal randomization of the oriented water molecules is resisted by their hydrogen bonding associations, thereby accounting for the high dielectric constant of water. Nonpolar substances are essentially insoluble in water. However, amphipathic substances aggregate in aqueous solutions to form micelles and bilayers due to the combination of hydrophobic interactions among the nonpolar portions of these molecules and the hydrophilic interactions of their polar groups with the aqueous solvent. The H_3O^+ and OH^- ions have anomalously large ionic mobilities in aqueous solutions because the migration of these ions through solution occurs largely via proton jumping from one H_2O molecule to another.

2 Acids, Bases, and Buffers A Brønsted acid is a substance that can donate protons, whereas a Brønsted base can accept protons. On losing a proton, a Brønsted acid becomes its conjugate base. In an acid–base reaction, an acid donates its proton to a base. Water can react as an acid to form hydroxide ion, OH^-, or as a base to form hydronium ion, H_3O^+. The strength of an acid is indicated by the magnitude of its dissociation constant, K. Weak acids, which have a dissociation constant less than that of H_3O^+, are only partially dissociated in aqueous solution. Water has the dissociation constant 10^{-14} at 25°C. A practical quantity for expressing the acidity of a solution is pH $= -\log[H^+]$. The relationship between pH, pK, and the concentrations of the members of its conjugate acid–base pair is expressed by the Henderson–Hasselbalch equation. An acid–base buffer is a mixture of a weak acid with its conjugate base in a solution that has a pH near the pK of the acid. The ratio $[A^-]/[HA]$ in a buffer is not very sensitive to the addition of strong acids or bases, so that the pH of a buffer is not greatly affected by these substances. Buffers are operationally effective only in the pH range of p$K \pm 1$. Outside of this range, the pH of the solution changes rapidly with the addition of strong acid or base. Buffer capacity also depends on the total concentration of the conjugate acid–base pair. Biological fluids are generally buffered near neutrality. Many acids are polyprotic. However, unless the pK's of their various ionizations differ by less than 2 or 3 pH units, pH calculations can effectively treat them as if they were a mixture of separate weak acids. For polyprotic acids with pK's that differ by less than this amount, the observed molecular ionization constants are simply related to the microscopic ionization constants of the individual dissociating groups.

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